

## Use of Chlorophyll Fluorescence Assessments to Differentiate Corn Hybrid Response To Variable Water Conditions

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### ABSTRACT

Development of corn (*Zea mays* L.) germplasm tolerant to water stress will be vital to sustaining corn-based farming in the U.S. Great Plains. In a companion 4-yr field study near Shelton, NE, we found that 12 hybrids displayed differential agronomic responses to varying water levels, with tolerant hybrids yielding from 27 to 42% more than susceptible hybrids under stress while yielding similarly under no stress. The objective of this study was to determine if chlorophyll fluorescence (CF) measurements could be used to distinguish tolerant from susceptible hybrids. Leaf temperature (LT) and two CF parameters ( $\Phi_{PSII}$ , photosystem II quantum efficiency, and ETR, electron transport rate) were measured on three postflowering dates in 2001 using a fluorometer on a subset of original treatments involving two tolerant and susceptible hybrids grown under deficit and adequate water. Water effects were observed on only one date; LT was 2.5°C warmer and  $\Phi_{PSII}$  and ETR values were 25% lower for deficit vs. adequate water just after silking, signifying increased water stress and decreased photosynthesis during reproductive growth. Under stress, LTs were 2.8°C cooler and  $\Phi_{PSII}$  and ETR values 50% higher for tolerant vs. susceptible hybrids, while all hybrids produced similar CF values under no stress. Thus, grain yield and photosynthetic responses of hybrids to stress were similar, indicating that CF measurements can be used to distinguish tolerant from susceptible hybrids.

IN COMPANION WORK (O'Neill et al., 2004), we noted the significant role that water and N fertilizer inputs have played in increasing productivity of corn grown in the Great Plains region of the USA. However, continued overuse of these inputs required to sustain current productivity poses serious environmental threats (Council for Agricultural Science and Technology, 1999). To minimize input costs and environmental impact, farmers will likely have to resort to producing corn with less irrigation water and fertilizer N in the future. This will lead to increased levels of water and N stress imposed on the crop. Development of corn hybrids tolerant to water and N stresses will be crucial to sustaining corn-based farming in the Great Plains region of the USA. Hence, future corn breeding efforts should focus on identifying physiological mechanisms that can be used to further improve tolerance of corn to these and other stresses.

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In the companion work we found that 12 hybrids displayed differential agronomic responses to varying levels of water and N. For example, under either limited water or N, stress tolerant hybrids yielded from 27 to 42% more than susceptible hybrids, while these same hybrids yielded similarly under adequate water and N levels. Furthermore, variation in hybrid yields under deficit water was better predicted by hybrid yields under deficit N than under adequate water conditions. Finally, variation in hybrid grain yields grown under varying water and N levels was strongly associated with hybrid variation in kernel number per unit area. Collectively, these results imply that water and N stresses produced similar adverse effects on key physiological processes, and hybrids possessing physiological mechanisms conferring ability to maximize kernel number under either water or N stress were critical to their ability to produce high grain yields. Other researchers have shown that kernel number is strongly linked to assimilate supply during the critical period around flowering (Schussler and Westgate, 1995).

Tollenaar and Aguilera (1992) confirmed the role of achieving high photosynthetic rates by showing that observed differences in dry matter accumulation between old and new hybrids were due to higher photosynthetic rates after silking for newer hybrids. Others (Sanchez et al., 1983; Wolfe et al., 1988; Dwyer et al., 1992; Aguilera et al., 1999) have noted that while stress reduces photosynthesis, the degree of reduction appears to vary among genotypes. Thus, we hypothesized that corn hybrids tolerant to drought would, for example, maintain higher photosynthetic rates compared to susceptible hybrids during this critical reproductive growth period, and photosynthetic assessments during this time may offer a potential means for identifying stress tolerant germplasm.

While measurements of leaf photosynthetic rates may offer promise for characterizing hybrid responses to stress, previous methods of assessing photosynthesis via gas exchange techniques have proven to be laborious and not practical in crop improvement programs (Earl and Tollenaar, 1999). Alternatively, CF techniques may serve as a more practical means for indirectly assessing leaf photosynthetic rates (Earl and Tollenaar, 1998; Adams et al., 2000; Jiang and Huang, 2000; Garty et al., 2001; Ying et al., 2002; Earl and Davis, 2003). Since each CF measurement requires only a few seconds, hundreds of measurements can be made per day with a single instrument, thus greatly improving on the

**Abbreviations:** CF, chlorophyll fluorescence; DAP, days after planting; ET, evapotranspiration; ETR, electron transport rate; LT, leaf temperature; PPFD, photosynthetic photon flux density;  $\Phi_{PSII}$ , photosystem II quantum efficiency.

**Table 1.** Mean grain yields and average (across both N levels) yield response to water for two “drought tolerant” and two “susceptible” corn hybrids after O'Neill et al. (2004). Means represent averages across four growing seasons (1999–2002) at Shelton, NE.

| Hybrid             | Era         | Water treatment         |          | Response‡ |
|--------------------|-------------|-------------------------|----------|-----------|
|                    |             | Deficit                 | Adequate |           |
|                    |             | — Mg ha <sup>-1</sup> — |          | %         |
| <b>Tolerant†</b>   |             |                         |          |           |
| B73 × Mo17         | 1970s       | 6.10                    | 7.20     | 24        |
| 3417               | Early 1990s | 6.75                    | 7.93     | 21        |
| Mean               |             | 6.43a                   | 7.57a    | 23b       |
| <b>Susceptible</b> |             |                         |          |           |
| 3162               | Early 1990s | 5.30                    | 7.61     | 50        |
| 33H67              | Late 1990s  | 5.98                    | 7.63     | 29        |
| Mean               |             | 5.64b                   | 7.62a    | 40a       |

† Tolerant and susceptible hybrid means within a column and having the same letter are not significantly different (0.05 probability level) as determined by single degree of freedom comparisons.

‡ Yield response to water within a given replication was calculated as: yield of adequate water minus yield of deficit water divided by yield of deficit water and multiply by 100 for each hybrid × N treatment combination for each replication. Water responses shown in this column represent average values across both levels of N and across all four study years.

sampling resolution that can be achieved over gas exchange techniques (Earl and Tollenaar, 1999). The objective of this study was to determine if CF assessments could be used to differentiate hybrid photosynthetic responses to variable water levels and distinguish stress tolerant from susceptible hybrids.

## MATERIALS AND METHODS

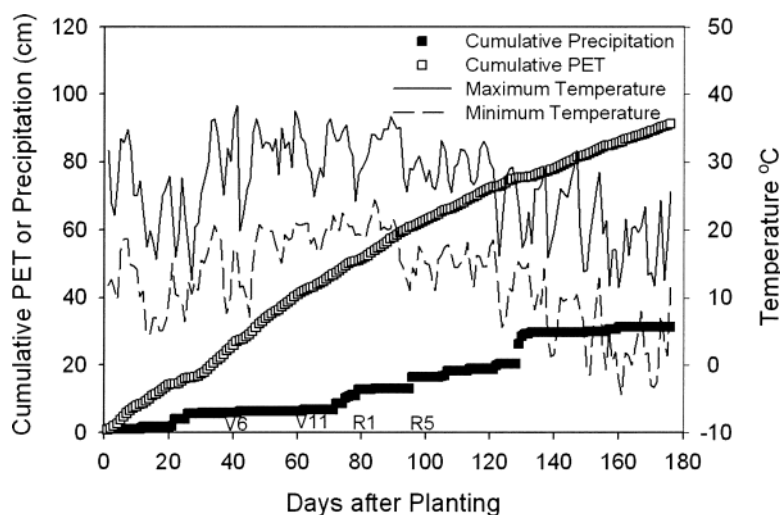
### Experimental Treatments

The CF measurements reported in this work were collected during the 2001 growing season from our ongoing companion field study conducted from 1999 through 2002 near Shelton, NE (40°45'01" N; 98°46'01" W, elevation 620 m above mean sea level). The objective of the companion study was to characterize agronomic responses of hybrids of different eras to varying water and N supply. Details related to crop cultural practices and experimental procedures are reported in the companion paper (O'Neill et al., 2004). Although treatments

in the companion study consisted of a factorial combination of 12 corn hybrids receiving deficit and adequate levels of both irrigation water and N, CF measurements were collected in this study only on 8 of the original 48 treatment combinations, involving four hybrids grown under deficit and adequate water levels (1/2 and full evapotranspiration [ET]) with adequate N. This was done to maximize sampling resolution within individual hybrids as well as collect measurements between 1100 and 1300 h (2-h time window) to minimize diurnal variation in photosynthetic photon flux density (PPFD) during sampling and its resultant effect on CF parameters. For example, Earl and Tollenaar (1999) and Earl and Davis (2003) observed that CF parameters were correlated with diurnal variation in PPFD during CF measurements. We selected the hybrids B73 × Mo17 and Pioneer Hybrids 3417, 3162, and 33H67 for the present study based on preliminary results we observed from the first 2 yr (1999–2000) of the field study, which indicated a significant differential yield response to varying water levels for the four hybrids. These results were confirmed for all 4 yr of the field study (Table 1). The differential yield response is apparent by noting that all four hybrids yielded similarly under adequate water, but under deficit water B73 × Mo17 and 3417 as a group yielded 14% more than 33H67 and 3162. Because B73 × Mo17 and 3417 exhibited less response to added water and yielded more under deficit water than 33H67 and 3162, the former were considered more “drought tolerant” than the latter two. Collectively, the four represented suitable candidates for determining if CF measurements could be used to differentiate hybrid photosynthetic responses to water stress.

The experimental design was a strip-split plot design, with water levels as whole plots, N levels as split plots, and corn hybrids as strip plots with three replications. The plots were seeded on 5 May 2001 (day of year = 129). Daily climatological data (Fig. 1) were recorded throughout the growing season using an automated weather station (High Plains Climate Center Network, University of Nebraska) located on the research site. Phenology data according to Ritchie et al. (1997) were recorded weekly from the first of June through mid-August.

Water treatments (deficit and adequate) were initiated during vegetative growth stage (around V9). Beginning on these dates water was applied at weekly intervals based on the amount of ET for the previous week as determined by the on-site weather station using a modified version of the Penman equation (Kincaid and Heerman, 1974). The adequate water



**Fig. 1.** Cumulative potential evapotranspiration (PET) and precipitation along with daily maximum and minimum temperatures vs. days after planting for the 2001 growing season at Shelton, NE. Important phenological growth stages are depicted near the bottom portion of graph. The R1 and R5 stages correspond to the silking and dent growth stages, respectively.

treatment received the amount of water required to fully replace the previous week ET while the deficit treatment received approximately one-half this amount. This was continued throughout the remainder of the growing season.

### Leaf Measurements

Measurements of leaf temperature, chlorophyll content, and CF parameters were made on three cloud free dates (83, 90, and 98 d after planting) in 2001 during the postflowering period. Readings were collected between approximately 1100 and 1300 h using a PAM-2000 fluorometer equipped with a fiber optic probe and 2030-B leaf clip holder (Heinz Walz GmbH, Effeltrich, Germany). On each measuring date, 15 individual plants were sampled from each of the 24 plots using sunlit ear leaves. This resulted in the collection of a total of 360 readings during the 2-hr sampling period for each sampling date. Care was taken before and during the measurement not to alter the natural leaf orientation with respect to the sun or to shade the tissue to be measured. Measurements were taken midway between the leaf tip and base and midway between the margin and the midrib of the leaf from representative plants of center two rows of each plot. Plants having unusual spacing or those that were damaged were not sampled. The fiber optic probe of the PAM-2000 used to detect CF was held at a 60° angle to the leaf tissue by the leaf clip attachment. Leaf temperature was recorded through the use of a NiCr–Ni thermocouple junction (0.1-mm diameter) located on the leaf clip holder, positioned to sample the underside of the leaf.

The sampling procedures and fluorometer settings for CF measurements used were as suggested by Earl and Tollenaar (1999). Briefly, the 20 kHz measuring light modulation frequency was used, with gain, damping, and measuring light intensity set to levels 4, 2, and 12, respectively. Steady state fluorescence ( $F_s$ ) was measured first, followed by exposure to a saturating pulse of light ( $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 0.8 s using the instrument's halogen source, to allow the maximum fluorescence ( $F_m$ ) to be determined. The microquantum sensor (1.5-mm diameter) located on the leaf clip holder recorded the PPFD incident to the leaf during  $F_s$  determination. The quantum efficiency of photosystem II ( $\Phi_{\text{PSII}}$ ) was calculated according to Genty et al. (1989) as  $\Phi_{\text{PSII}} = (F_m - F_s)/F_m$ , and corresponds to the efficiency with which photons absorbed by the chlorophyll of photosystem II are used to carry out charge separation in the reaction center. This value is used to determine electron transport rate when PPFD and the allocation of photons to photosystem II are known (Genty et al., 1989). Because photorespiration in  $C_4$  species like corn is minimal, the ETR is closely linked with the gross  $\text{CO}_2$  assimilation rate (Edwards and Baker, 1993; Earl and Tollenaar, 1998). Thus, at any given PPFD level,  $\Phi_{\text{PSII}}$  can be directly related to gross  $\text{CO}_2$  assimilation in corn.

At the same time that CF was determined, SPAD readings were also taken on 15 leaves per plot using a SPAD 502 Chlorophyll Meter (Minolta Corporation, Ramsey, NJ). These leaves were chosen using the same criterion as for CF measurements. SPAD readings are based on measurements of transmittance of red and far red light through the leaf and in corn are strongly associated with leaf absorbance of PAR (Earl and Tollenaar, 1997). The mean SPAD value for each subplot on each measuring day was used to estimate leaf absorbance of incident PPFD, using the method described by Earl and Tollenaar (1997), with absorbance or  $\alpha_L = 0.409 + 0.528(1 - e^{-0.0429\text{SPAD}})$ . ETR was calculated for each sample as described by Earl and Tollenaar (1999):  $\text{ETR} = \alpha_L f_{\text{II}} \Phi_{\text{PSII}}$  PPFD, using the SPAD-derived  $\alpha_L$  from each plot on that day and assuming a value of 0.4 for  $f_{\text{II}}$ , the fraction of PPFD ab-

sorbed by photosystem II (Edwards and Baker, 1993). Although Earl and Tollenaar (1999) and Earl and Davis (2003) observed associations between PPFD vs.  $\Phi_{\text{PSII}}$ , we did not detect an association (linear or nonlinear) on any measurement date, when examining relationships of within subplot values for PPFD vs.  $\Phi_{\text{PSII}}$  for each hybrid by water treatment combination. The lack of association between PPFD and CF parameters in our work is difficult to explain. Perhaps, it was due to the relatively narrow time window (between 1100 and 1300 h) that sampling occurred in our work and the relatively small range in PPFD values ( $1100\text{--}1700 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) during measurements. It should also be noted that the ANOVA (not shown) for PPFD revealed no effect from water or hybrid treatments or their interaction on any measurement date. Thus, the variation in PPFD during CF measurements was not biased in favor of a particular hybrid or water treatment combination. Hence, the 15 individual CF measurements for each plot were simply averaged, without any adjustment for PPFD, to produce one CF value per plot.

### Statistical Analysis

Analysis of variance for each physiological variable was performed with the SAS PROC MIXED procedure (Littell et al., 1996), using the Kenward–Roger degrees of freedom method. This method uses an adjusted estimator of the covariance matrix to reduce small sample bias (Kenward and Roger, 1997). Hybrids and water treatments were considered fixed effects, replication random effects, and measurements dates as repeated observations in the analysis. Individual hybrid means were compared using LSD values and drought tolerant vs. susceptible groups compared using single degree of freedom contrasts. Linear correlation analysis was used to determine the associations between leaf temperature, chlorophyll,  $\Phi_{\text{PSII}}$ , and ETR on each measurement date.

## RESULTS AND DISCUSSION

### Climatological Conditions

Seasonal air temperatures and precipitation received during 2001 (Fig. 1) were near long-term averages for this location. Comparing accumulated potential ET and precipitation across the growing season underscores the drought prone nature of this environment. For example, accumulated ET was four to five times greater than precipitation received during the measurement period between 80 and 100 d after planting (DAP). No precipitation was received during the entire measurement period, except for a 3-mm event that occurred 1 d before the final date (98 DAP) (Fig. 1). Thus, climatic conditions were conducive to the development of differences in stress between water treatments (initiated around 60 DAP) during the postflowering period, providing a suitable environment to successfully address the study objectives.

### Water Treatment Effects on Leaf Measurements

Leaf temperatures acquired simultaneously with CF measurements (via the thermocouple attached to the leaf measurement clip) were used to determine the impact of water treatments on plant stress. Leaf temperature is considered to be a proven indicator of plant water stress (Tanner 1963; Clark and Hiler, 1973; Ehrler



**Table 2. Analysis of variance for leaf temperature, chlorophyll content (via SPAD chlorophyll meter), photochemical quantum efficiency of photosystem II ( $\Phi_{PSII}$ ), and electron transport rate (ETR) for four corn hybrids grown under deficit and adequate water levels and measured on three dates in 2001.**

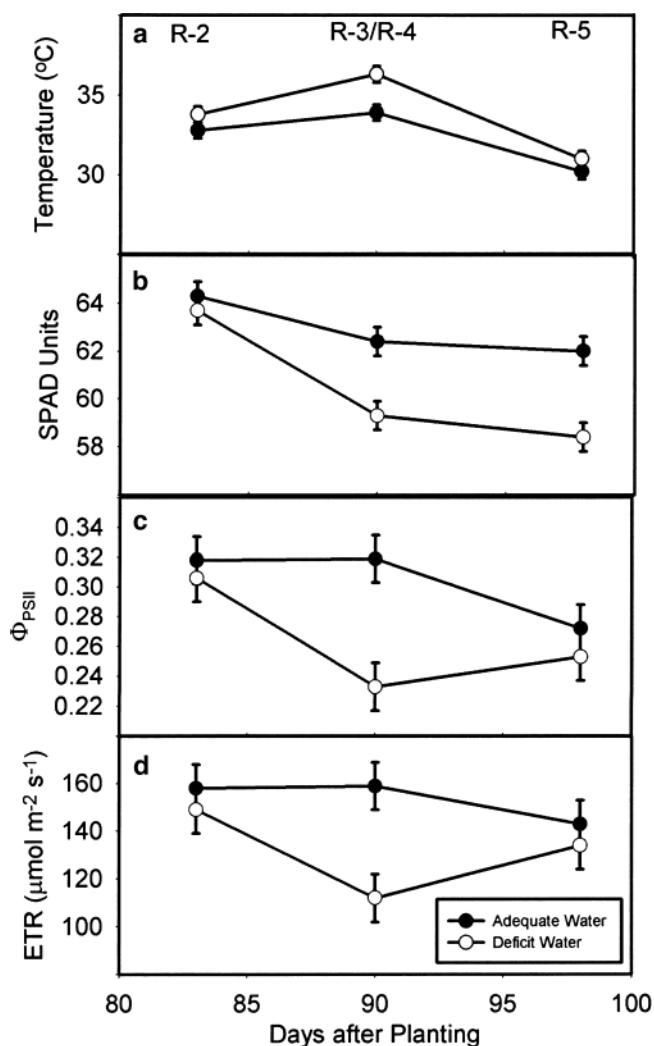
| Source of variation     | df | Physiological variables |             |               |        |
|-------------------------|----|-------------------------|-------------|---------------|--------|
|                         |    | Temperature             | Chlorophyll | $\Phi_{PSII}$ | ETR    |
|                         |    | $P > F$                 |             |               |        |
| Water (W)               | 1  | 0.0459                  | 0.0364      | 0.0583        | 0.1767 |
| Date (D)                | 2  | <0.0001                 | <0.0001     | 0.0040        | 0.0389 |
| W $\times$ D            | 2  | 0.0452                  | 0.0833      | 0.0264        | 0.0126 |
| Hybrid                  | 1  | 0.1603                  | 0.2202      | 0.0082        | 0.2152 |
| group (H)               |    |                         |             |               |        |
| W $\times$ H            | 1  | 0.0130                  | 0.1027      | 0.0013        | 0.0012 |
| H $\times$ D            | 2  | 0.0463                  | 0.4347      | 0.0257        | 0.0141 |
| W $\times$ H $\times$ D | 2  | 0.1104                  | 0.2266      | 0.2593        | 0.1270 |

et al., 1978; Sumayao and Kanemasu, 1979; Jackson et al., 1981; Raskin and Ladyman, 1988), and is based on the principle that increasing plant water deficits lead to stomatal closure, decreased leaf transpirational cooling, and consequently increased leaf temperature relative to well-watered plants. The ANOVA (Table 2) for leaf temperature revealed a significant water treatment  $\times$  date interaction, which is illustrated in Fig. 2. The interaction was due to a lack of significant temperature differences between water treatments on the first (83 DAP) and third sampling dates (98 DAP); whereas, on the second date (90 DAP) average temperatures were approximately 2.5°C warmer for the deficit vs. adequate water level, indicating greater plant water stress for the deficit vs. adequate water level. Apparently, climatic conditions were most favorable for manifestation of differences in stress between water treatments on the second sampling date.

Responses of photosynthetic indicators (SPAD-derived chlorophyll content,  $\Phi_{PSII}$ , and ETR) to water levels were in general similar to leaf temperature responses, and varied with sampling date (Table 2). Most of the significant differences between water treatments existed on the second sampling date, when chlorophyll content was reduced approximately 5%, and  $\Phi_{PSII}$  and ETR values 30% for the deficit vs. adequate water level (Fig. 2). On the final date, leaf chlorophyll content was the only variable affected by water treatments, with SPAD readings reduced by 6% for the deficit vs. adequate water level. Since  $\Phi_{PSII}$  and ETR are direct indicators of gross  $CO_2$  assimilation rates for corn (Edwards and Baker, 1993; Earl and Tollenaar, 1998), our data would indicate that water deficits reduced photosynthetic capacity of the crop only on the second measurement date. To evaluate relationships among leaf temperature, chlorophyll content,  $\Phi_{PSII}$ , and ETR across the three sampling dates, linear correlation matrices involving these four variables were computed for each sampling date, using mean values of the variables for each hybrid grown under both deficit and adequate water levels on each of the three dates (Table 3). It should be noted that correlations between  $\Phi_{PSII}$  and ETR were significant on all measurement dates, and this was because ETR was calculated from  $\Phi_{PSII}$  along with PPFD values. Although leaf temperatures tended to be negatively associated with chlorophyll content,  $\Phi_{PSII}$ , and ETR on all

measurement dates, the correlations were significant only on the last two dates and largest on the second date, with a maximum correlation of  $r = -0.945$  for leaf temperature vs.  $\Phi_{PSII}$ . Thus, treatment induced variation in photosynthetic indicators (leaf chlorophyll,  $\Phi_{PSII}$ , and ETR) was most negatively associated with variation in leaf temperatures on the second date, when differences in stress between water treatments were most pronounced (Fig. 2).

The observation that leaf temperatures were most negatively associated with leaf photosynthetic indicators (chlorophyll content,  $\Phi_{PSII}$ , and ETR), when water stress was most pronounced (Fig. 2 and Table 3), is not surprising, given the direct effect that plant water deficits have on physiological attributes like stomatal conductance, transpiration, and photosynthesis. For example, stomatal closure is one of the first adaptive responses plants display to increasing water stress, in an attempt to reduce leaf transpirational losses and prevent



**Fig. 2. Average leaf temperature (a), chlorophyll content via SPAD chlorophyll meter (b), quantum efficiency of photosystem II,  $\Phi_{PSII}$  (c), and thylakoid electron transport rate, ETR (d) for two water treatments (deficit and adequate) on three measurement dates in 2001 at Shelton, NE. Phenological growth stages for three measurement dates are also shown in the upper portion of graph.**

**Table 3. Genotypic correlation coefficients for associations among leaf temperature, chlorophyll content (via SPAD chlorophyll meter), photochemical quantum efficiency of photosystem II ( $\Phi_{PSII}$ ), and electron transport rate (ETR), using means values ( $n = 8$ ) of four corn hybrids grown under two water treatments (deficit and adequate) measured on three different dates in 2001.**

|                               | Temperature | Chlorophyll | $\Phi_{PSII}$ | ETR   |
|-------------------------------|-------------|-------------|---------------|-------|
| <b>83 Days after planting</b> |             |             |               |       |
| Temperature                   | 1.000       |             |               |       |
| Chlorophyll                   | -0.427      | 1.000       |               |       |
| $\Phi_{PSII}$                 | -0.270      | -0.564      | 1.000         |       |
| ETR                           | -0.324      | -0.670      | 0.869**       | 1.000 |
| <b>90 Days after planting</b> |             |             |               |       |
| Temperature                   | 1.000       |             |               |       |
| Chlorophyll                   | -0.787*     | 1.000       |               |       |
| $\Phi_{PSII}$                 | -0.945**    | 0.639       | 1.000         |       |
| ETR                           | -0.935**    | 0.573       | 0.993**       | 1.000 |
| <b>98 Days after planting</b> |             |             |               |       |
| Temperature                   | 1.000       |             |               |       |
| Chlorophyll                   | -0.427      | 1.000       |               |       |
| $\Phi_{PSII}$                 | -0.794*     | 0.032       | 1.000         |       |
| ETR                           | -0.759*     | -0.020      | 0.995**       | 1.000 |

\* Indicates significance at 0.05 level.

\*\* Indicates significance at 0.01 level.

development of lethal water deficits in their tissues (Kramer and Boyer, 1995). The elevated leaf temperature we observed for the deficit vs. adequate water level on the second date (Fig. 2) confirmed that stomatal aperture was reduced most by water deficit on the second measurement date. Stomatal closure in turn negatively impacts photosynthesis, as it increases resistance to diffusion of  $CO_2$  into internal leaf tissue where it is assimilated in the photosynthetic process (Farquhar and Sharkey, 1982). Our CF assessments of photosynthesis confirmed the negative impact of stomatal closure on photosynthetic rates on the second date (Fig. 2). Hence, the strong negative associations (Table 3) we observed between leaf temperatures and CF-determined photosynthetic indicators under pronounced water deficit conditions are consistent with well-documented physiological responses that plants exhibit to increasing water deficits. Other researchers (Lu and Zhang, 1998; Tambussi et al., 2002; Colum and Vazzana, 2003; Earl and Davis, 2003) have also shown that CF measurements can be used to verify the adverse effects of plant water deficits on photosynthetic rates for several different plant species including corn.

Although there were no differences between water treatments for leaf temperature or CF variables on the third sampling date (Fig. 2), indicating water stress was relieved relative to the previous date, SPAD values were 6% lower for the deficit vs. adequate water level, suggesting leaf chlorophyll content had not recovered from water stress occurring on the prior sampling date. However, it should be noted that SPAD readings are known to underestimate actual leaf chlorophyll content for water-stressed corn leaves (Schlemmer et al., 2005). Nonetheless, our leaf measurements indicated increased water stress and severe reductions in leaf photosynthesis on the second sampling date, which coincided with the critical R3 growth stage. Schussler and Westgate (1995), Andrade et al. (2002), and Bänziger et al. (2002) have in turn shown that kernel number and grain yield are extremely sensitive to reductions in photosynthesis and assimilate levels during this time. Thus, the yield losses we reported for the deficit vs. adequate water treatment in our companion work (O'Neill et al., 2004), and illustrated in Table 1, were likely due to reductions in photosynthesis during this critical growth stage.

### Hybrid Responses to Plant Water Stress

To address the study objective of whether CF assessments could be used to differentiate hybrid photosynthetic responses to variable water, the water  $\times$  hybrid interaction terms for leaf temperature, chlorophyll content,  $\Phi_{PSII}$ , and ETR were evaluated (Table 2). Significant interactions were observed for all variables except SPAD-determined chlorophyll content. The differential hybrid response to water was most prominent on the second measurement date, when water stress was most pronounced (Fig. 2), and is illustrated in Table 4. Under adequate water, the tolerant and susceptible hybrid groups produced similar leaf temperature, chlorophyll,  $\Phi_{PSII}$ , and ETR values. However, under deficit water, leaf temperatures were 2.8°C cooler while  $\Phi_{PSII}$  and ETR values were 50% higher for tolerant vs. susceptible hybrids. Although hybrids displayed somewhat similar photosynthetic responses to variable water conditions on the other two measurement dates (data not shown), the differences between tolerant and susceptible hybrids under deficit water were not significant. Collectively, these results indicate that CF assessments

**Table 4. Leaf temperature, chlorophyll (via SPAD chlorophyll meter), photochemical quantum efficiency of photosystem II ( $\Phi_{PSII}$ ), and electron transport rate (ETR) on 90 d after planting for four corn hybrids grown under deficit (-W) and adequate (+W) water levels in 2001.**

| Hybrid            | Temperature |        | Chlorophyll |        | $\Phi_{PSII}$ |         | ETR                                  |       |
|-------------------|-------------|--------|-------------|--------|---------------|---------|--------------------------------------|-------|
|                   | -W          | +W     | -W          | +W     | -W            | +W      | -W                                   | +W    |
| Tolerant          | °C          |        | SPAD units  |        |               |         | $\mu\text{mol m}^{-2} \text{s}^{-1}$ |       |
| 3417              | 35.0        | 34.3   | 57.8        | 60.8   | 0.314         | 0.336   | 159                                  | 169   |
| B73 $\times$ Mo17 | 34.7        | 33.5   | 63.8        | 63.4   | 0.297         | 0.317   | 139                                  | 155   |
| Mean              | 34.9 b†     | 33.9 a | 60.8 a      | 62.1 a | 0.305 a       | 0.326 a | 149 a                                | 162 a |
| Susceptible       |             |        |             |        |               |         |                                      |       |
| 3162              | 37.5        | 34.0   | 57.1        | 61.3   | 0.153         | 0.315   | 75                                   | 151   |
| 33H67             | 37.8        | 33.6   | 58.3        | 63.9   | 0.167         | 0.309   | 74                                   | 171   |
| Mean              | 37.7 a      | 33.8 a | 57.7 b      | 62.6 a | 0.160 b       | 0.312 a | 74 b                                 | 161 a |

† Tolerant and susceptible hybrid means within a column and having the same letter are not significantly different (0.05 probability level) as determined by single degree of freedom comparisons.

can be used to detect differential photosynthetic responses of hybrids to variable water levels, provided that the plants are exposed to deficit water conditions. Tollenaar and Wu (1999) also concluded that genetic differences in stress tolerance among hybrids are only expressed under stressful conditions.

Subsequent to determining whether CF assessments could be used to differentiate photosynthetic responses of hybrids to variable water, we were also interested in knowing if these measurements could be used to distinguish drought tolerant from susceptible hybrids. As previously mentioned, the two tolerant and susceptible hybrids used for CF measurements in this study were chosen based on their differential agronomic responses to variable water. In the previous work, the yield advantage of tolerant hybrids (Table 1) was attributed to the ability to maintain higher kernel number than susceptible hybrids under stress conditions, as variation in kernel number was highly correlated ( $r = 0.985$ ) with grain yield variation. Other researchers (Schussler and Westgate, 1995) have in turn shown that ability to maintain photosynthesis and assimilate supply under stress during and after flowering are crucial for maintaining seed number and grain yield. Thus, we hypothesized that the tolerant hybrids we evaluated would maintain higher photosynthetic rates compared to susceptible hybrids during the critical reproductive growth period conferring the ability to maximize kernel number and yield under stress. Since the photosynthetic and agronomic responses of the two hybrid groups are similar (Tables 1 and 4), it would appear that CF assessments of photosynthesis could in fact be used to distinguish tolerant from susceptible hybrids.

## SUMMARY AND CONCLUSION

Using a PAM-2000 fluorometer, leaf temperature and CF indicators  $\Phi_{PSII}$  and ETR were measured for two drought tolerant and two susceptible hybrids grown under deficit and adequate water levels on three postflowering dates in 2001 to determine if these measurements could be used to differentiate hybrid photosynthetic responses to postflowering stress. Effects on measured variables were observed on one of three dates, with average leaf temperature 2.5°C warmer and  $\Phi_{PSII}$  and ETR values 25% less for deficit vs. adequate water level during the critical R3 reproductive growth stage, indicating water stress was increased and photosynthesis decreased for deficit water conditions. Under deficit water, leaf temperatures were 2.8°C cooler and  $\Phi_{PSII}$  and ETR values 50% higher for tolerant vs. susceptible hybrids, while all hybrids produced similar leaf temperatures,  $\Phi_{PSII}$ , and ETR values under no stress. Thus, agronomic (Table 1) and photosynthetic (Table 4) responses of the hybrids to deficit and adequate water were similar, indicating that CF measurements can be used to distinguish tolerant from susceptible hybrids. While Earl and Tollenaar (1999) indicated that fluorometry would be more practical than gas exchange measurements for assessing crop photosynthetic performance, Baker and Rosenqvist (2004) suggested that the

recently developed technique of imaging CF signals using charge-coupled camera devices would be an even more attractive means for accomplishing this goal. In their review of CF literature they examined the potential role of using CF imaging in increasing both the sensitivity and throughput of screening plants for tolerance to environmental stresses. Alternatively, the approach proposed by Zarco-Tejada et al. (2002) of using remotely sensed hyperspectral imagery along with use of a fluorescence–reflectance–transmittance leaf model to estimate parameters like  $\Phi_{PSII}$  may also hold promise for mapping differences in canopy stress among various genotypes grown under field stress conditions.

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